A STUDY OF HAEMATOLOGICAL PROFILE AND SERUM IRON INDICES IN NONDIALYSIS CHRONIC KIDNEY DISEASE PATIENTS

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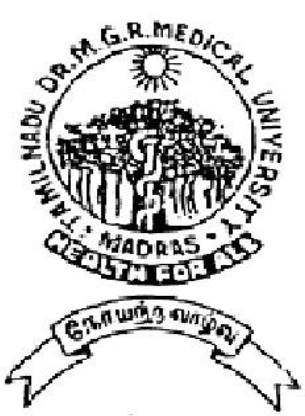

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CERTIFICATE

This is to certify that this dissertation titled **"A STUDY OF HAEMATOLOGICAL PROFILE AND SERUM IRON INDICES IN NONDIALYSIS CHRONIC KIDNEY DISEASE PATIENTS"** submitted by **DR. KANNAN. N** to the faculty of General Medicine, The Tamil Nadu Dr.M.G.R. Medical University, Chennai in partial fulfillment of the requirement for the award of MD degree Branch I (General Medicine) is a bonafide research work carried out by him under our direct supervision and guidance.

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DECLARATION

I, **Dr. KANNAN. N** solemnly declare that the dissertation titled **'A STUDY OF HAEMATOLOGICAL PROFILE AND SERUM IRON INDICES IN NONDIALYSIS CHRONIC KIDNEY DISEASE PATIENTS'** has been prepared by me at the Department of General Medicine, Govt. Rajaji Hospital, Madurai, under the guidance of **Dr. R. Balajinathan, M.D** Professor of Medicine, Department of General Medicine, Madurai Medical college, Madurai.

This dissertation is submitted to The Tamil Nadu Dr. M. G. R. Medical University, Chennai in partial fulfillment of the rules and regulations for the award of M.D Degree General Medicine Branch-I examination to be held in April 2012.

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CONTENTS

GLOSSARY

CHF - Congestive Heart Failure

CKD - Chronic Kidney Disease

CHr - Reticulocyte haemoglobin content

CBC - Complete blood count

CRP - C reactive protein

ESA - Erythropoiesis stimulating agents

ESR - Erythrocyte sedimentation rate

ESRD - End Stage Renal Disease

GFR - Glomerular Filtration Rate

HD - Haemodialysis

Hb - Hemoglobin

HIV - Human Immunodeficiency Virus

HCV - Hepatitis C Virus

LVH - Left Ventricular Hypertrophy

NKF -K/DOQI- Kidney Disease Outcomes Quality Initiative of National Kidney Foundation

NSAID- Non steroidal anti-inflammatory drugs

MCV - Mean corpuscular volume

MCH - Mean corpuscular hemoglobin

MCHC - Mean corpuscular hemoglobin concentration

PCV - Packed cell volume

PD - Peritoneal Dialysis

RBC - Red blood cell

rHuEPO- recombinant human erythropoietin

RES - Reticuloendothelial system

RDW - Red cell distribution width

sTfr - Soluble transferrin receptor

TIBC - Total iron binding capacity

WBC - White blood cell count

%TSAT- Percentage transferrin saturation

ABSTRACT

TITLE : A study of Haematological profile and Serum Iron indices in non-dialysis Chronic Kidney Disease patients

AIM : To assess the Haematological profile and Serum Iron indices in non-dialysis Chronic Kidney Disease patients

BACKGROUND AND OBJECTIVES: Anemia is a common and early complication of chronic kidney disease patients. One contributing factor is iron deficiency, which may be particularly problematic during erythropoietin therapy.

MATERIALS AND METHODS: It is a cross-sectional study conducted in Department of Medicine and Nephrology, Govt Rajaji Hospital, Madurai. A total of 54 patients were included in our study who satisfied the diagnostic criteria of CKD and patients underwent clinical and renal parameters, haematological profile and iron status. For comparison of the results with the general population adequate number of controls were taken.

RESULTS: Our study results showed low level of Haemoglobin, and packed cell volume with increase in severity of chronic kidney disease. Bleeding time was increased in 5.6% patients and elevated ESR was present in more than half of patients. Anemia was universal in our population. Normocytic normochromic anemia was found in 70.4 % of the patients and microcytic hypochromic anemia in another 20.4%, and 9.2% had both type of peripheral smear picture. Applying the NKF-K/DOQI guidelines for nondialysis chronic kidney disease to our population it was found that nearly 38.9% of the study population did not have target serum ferritin of 100 ng/ml and 44.4% of study population did not have target TSAT of >20%.

CONCLUSION: So it is vital to address this issue of iron deficiency in patients with chronic kidney disease so that necessary investigations can be undertaken to find the cause of iron deficiency if any. So every effort should be done to identify the cause of anemia in CKD patients and treat the coexistent iron deficiency anemia in chronic kidney disease patients. And other haematological parameters should be monitored to find out coexisting abnormality.

INTRODUCTION

Chronic kidney disease (CKD) encompasses a spectrum of various pathophysiologic processes leading to abnormal kidney function, and a progressive decline in glomerular filtration rate (GFR).

The burden of chronic kidney disease cannot be assessed accurately. The approximate prevalence of CKD is 800 per million population, and the incidence of end stage renal disease (ESRD) is 150-200 per million population.

CKD is a worldwide epidemic associated with a number of co-morbidities and hence a disease with high mortality. [1, 2]

Anemia of chronic disease is a complex disorder determined by variety of factors. Although the primary defect is decreased erythropoietin production from the kidney, a number of other factors may play contributory roles. For example iron, folate, vitamin B12 deficiency due to nutritional insufficiency or increased blood loss, shortened RBC survival, hyperparathyroidism, mild chronic inflammation and aluminium toxicity. Anemia in CKD worsens co-morbidities of diabetes and hypertension, contributing to poor outcome and high mortality.

Untreated chronic anemia leads to a number of physiologic disorders including cardiovascular complications and increased mortality and morbidity. According to GFR and 2006 NKF-K/DOQI guidelines,

CKD has been divided into 5 stages [3, 4]. Anemia usually appears at GFR below 60ml/min or at stage 3.

Renal insufficiency is also associated with bleeding tendency attributed to platelet dysfunction due to abnormal platelet aggregation and adhesiveness[5, 6]. White blood cell count may be decreased in uremic patients and anemia correction is followed by an increase in natural killer cells and improvement in leukocyte phagocytic function. Early identification and treatment of anemia in CKD may improve cardiovascular morbidity and mortality.[7] Early treatment of anemia in CKD may postpone the onset of ESRD and improve survival.

The identification, evaluation and optimal treatment of anemia in CKD essentially involve complete blood count, determination of serum ferritin and transferrin saturation to assess iron stores and adequacy of iron for erythropoeisis.

The National Kidney Foundation's Kidney Disease Outcome Quality Initiative (K/DOQI) anemia guidelines recommend that during erythropoiesis-stimulating agent (ESA) treatment in nondialysis CKD that serum ferritin and transferrin saturation (TSAT) be maintained >100 ng/ml and 20%, respectively [8]

Treatment of anemia in CKD when indicated may involve iron therapy, use of erythropoietin, and correction of anemia to a target hemoglobin of 11- 12 gm /dl.[8]

Renal replacement therapy poses a huge economic burden to the family and health care delivery system.

This study was conducted to determine the haematological profile and serum iron indices of non dialysis CKD patients.

AIM AND OBJECTIVES

AIM :

To assess haematological profile and serum iron indices in nondialysis chronic kidney disease patients.

Objectives :

1) To study the haematological profile and serum iron indices in non dialysis chronic kidney disease patients.

2) To detect the types of anemia in patients with chronic kidney disease

3) To study the prevalence of iron deficiency in non dialysis chronic kidney disease patients according to National Kidney Foundation's Kidney Disease Quality Initiative (NKF-K/DOQI) Guidelines.

REVIEW OF LITERATURE

CHRONIC KIDNEY DISEASE

Chronic kidney disease (CKD) has emerged as a major public health problem worldwide. It is well accepted that low income countries are unable to afford the cost of care required to manage patients with end stage renal disease.

The term chronic renal failure applies to the process of continuing significant irreversible reduction in nephron number, and typically corresponds to CKD stages 3-5.

The term end-stage renal disease represents a stage of CKD where the accumulation of toxins, fluids, and electrolytes that are normally excreted by the kidneys results in uremic syndrome. This syndrome leads to death unless the toxins are removed by renal replacement therapy, using dialysis or kidney transplantation.[4]

Chronic kidney disease is divided in to five stages based on the estimated GFR. To be classified as stage 1 or stage 2, there must be an accompanying structural or functional defect (eg.proteinuria, hematuria) as the GFR is normal or near normal in this stages.[2, 3]

DEFINITION OF CHRONIC KIDNEY DISEASE: [3]

National kidney foundation has defined CKD,

CRITERIA:

1. Kidney damage >3 months, either structural or functional abnormality with or without decreased GFR, manifested by either pathologic abnormalities or markers of kidney damage in blood, urine or imaging studies.

2. GFR <60 ml/min/1.73 sq m for > 3 months with or without kidney damage.

CLASSIFICATION OF CHRONIC KIDNEY DISEASE[3]:

STAGE	DESCRIPTION	GLOMERULAR FILTRATION RATE (ml/min/1.73 m^2)
0	With risk factor for CKD	>90
1	Kidney damage with normal or increased GFR	≥ 90
2	Mild decrease in GFR	60 – 89
3	Moderate decrease in GFR	30 – 59
4	Severe decrease in GFR	15 – 29
5	Kidney failure	< 15 or on dialysis

(Stage 0 – with risk factor for chronic kidney disease)

CALCULATION OF GFR [2, 4]

Recommended equation for estimation of GFR using serum creatinine, age, sex, and race & body weight.

Two formulas are used widely to estimate kidney function from serum creatinine: (1) Cockcroft-Gault and (2) four-variable MDRD (Modification of Diet in Renal Disease).

Cockcroft-Gault: CrCl (mL/min) = (140 – age (years) × weight (kg) × [0.85 if female])/ (72 × sCr (mg/dL)

MDRD: eGFR (mL/min per 1.73 m^2) = 186.3 × P_{Cr} ($e^{-1.154}$) × age ($e^{-0.203}$) × (0.742 if female) × (1.21 if black).

PATHOPHYSIOLOGY OF CHRONIC KIDNEY DISEASE:

The pathophysiology of CKD involves two broad sets of mechanisms of damage:

1) Initiating mechanisms specific to the underlying aetiology (e.g. Immune complex and mediators of inflammation in certain types of glomerulonephritis or toxin exposure in certain diseases of the renal tubules and interstitium)

2) A set of progressive mechanisms, involving hyperfiltration and hypertrophy of the remaining viable nephrons, that are a common consequence following long-term reduction of renal mass, irrespective of underlying aetiology. The responses to reduction in nephron number are mediated by vasoactive hormones, cytokines and growth factors.

Eventually these short term adaptations of hypertrophy and hyperfiltration become maladaptive as the increased pressure and flow predisposes to sclerosis and dropouts of the remaining nephrons.[4]

It is important to identify factors that increase the risk for CKD, even in individual with normal GFR.

Risk factors include:

1) Hypertension
2) Diabetes mellitus
3) Auto-immune disease
4) Older age
5) Structural abnormalities of urinary tract.
6) Family history of renal disease
7) A previous episode of acute renal failure
8) Presence of proteinuria
9) Abnormal urinary sediment

The most frequent cause of CKD is diabetic nephropathy most often secondary to type-2 diabetes mellitus. Hypertensive nephropathy is a common cause of CKD in elderly. Glomerulonephritis represents third most common cause of CKD. The early stage of CKD, manifesting as albuminuria and even a minor decrements in GFR, is now recognized as a major risk factor for cardiovascular disease. Other causes like interstitial

nephritis, HIV nephropathy, etc also form a significant proportion of cases leading to End Stage Renal Disease.[2, 4]

HAEMATOLOGICAL ASPECTS OF CHRONIC KIDNEY DISEASE:

ANEMIA:

Anemia is a common problem for CKD patients. The anemia of CKD is multifactorial in origin. (But erythropoietin deficiency is the most important etiologic factor). Even though traditionally considered as normochromic normocytic anemia due to erythropoietin deficiency other factors like iron deficiency contributes a major proportion and this is worsened in patients on dialysis. [9](Eschbach et al)

Anemia a multifactorial risk factor for the progression of CKD to end stage renal disease (ESRD) reduces the quality of life and associated with significant morbidity and mortality.

Anemia develops earlier in CKD among patients with diabetes mellitus and this magnitude of anemia tends to be more severe than with non diabetic patients [10]. (Mohanram A et al)

Degree of anemia is a reflection of severity of disease.

The WHO defines anemia as a haemoglobin level less than 13gm/dl in adult men and less than 12 gm/dl in adult women.

Absolute Hb level that defines anemia in CKD has been determined by National Kidney Foundation's kidney disease outcome quality initiative anemia guidelines as a level of less than 13.5gm/dl for men and 12gm/dl for women [8].

In general, anemia becomes more frequent as renal function declines, becoming almost universal in end-stage renal disease (ESRD). Hsu and co-workers studied 12,055 adult ambulatory subjects from health clinics in Boston, found that mean Haematocrit values decreased progressively when creatinine clearance was below 60 mL/min in men and below 40 mL/min in women. Moderately severe anemia, Haematocrit less than 33%, was common (present in >20% of patients) only when GFR was severely depressed, less than 30 mL/min in women and 20 mL/min in men. Erythropoietin deficiency along with absolute or functional deficiency of iron, accounts for nearly 90% cases of anemia. India is leading in iron deficiency anemia in the world. With or without CKD, anemia affects an estimated 2/3rd population in India, as per national family health survey [11]. (Anwer et al)

ETIOLOGY OF ANEMIA IN CKD:

BASIC ETIOLOGY:

1) Erythropoietin deficiency
2) Iron deficiency (absolute/functional)

- Decreased RBC life-span,
- Reduced food / iron intake & absorption due to uremia,
- Increased iron loss – GI bleeding, other bleeding tendency.
- Urinary loss of transferrin as a part of proteinuria leading to impaired iron transport.

CONTRIBUTORY FACTORS:

1) Uremic toxins
2) Drugs
3) Aluminium toxicity
4) Secondary hyperparathyroidism / bone marrow fibrosis
5) Folate / B12 deficiency
6) HIV / HCV infections
7) Chronic inflammation & cytokines
8) Hemoglobinopathy
9) Co-morbid conditions like auto-immune diseases, etc

CONSEQUENCES OF ANEMIA: [2, 11]

1) Decreased quality of life
2) Decreased exercise tolerance
3) Decreased cognitive functions
4) Left ventricular hypertrophy
5) Congestive heart failure

6) Angina / myocardial infarction

7) Disturbed sleep pattern

8) Decreased immune response

Impact of Anemia on Cardiac Health:

Cardiac disease has a grave impact on patients with kidney disease, reducing quality of life and increasing risk for hospitalizations and death. Among hemodialysis patients, mortality risk due to cardiovascular disease is more than 15 times greater than in the normal population. Approximately 50% of deaths in CKD are related to cardiovascular disease, owing to congestive heart failure (CHF), acute myocardial infarction, and sudden cardiac death.[12] (Wali RK et al). Indeed, patients with CKD are far more likely to die of cardiac events than to progress to ESRD.[13] (Keith DS et al). Anemia, a common complication in CKD, may play a key role in incrementing risk.

Anemia in CKD results in chronic changes in the cardiovascular system. Part of the body's compensation for anemia is a high cardiac output and vasodilated state, which partially mitigates the effect of reduced oxygen carriage by the bloodstream. Chronic elevation of cardiac output may be maladaptive, increasing cardiac work and resulting in left ventricular hypertrophy and increased risk for cardiovascular events [13, 14].

ANEMIA AND LEFT VENTRICULAR HYPERTROPHY (LVH)

Left ventricular hypertrophy is the cardiac abnormality most often found in association with chronic anemia. It is readily diagnosed by characteristic echocardiogram findings, [14] with left ventricular mass index greater than 134 and 110 g/m^2 in men and women, respectively.[15] (Abergel E et al) It is a particularly important finding in that it is a strong independent predictor of mortality risk. Each 1 gm/dL decrease in Hemoglobin was associated with a 6% increase in risk for LVH.[16]

Taken together, this literature indicates a fairly consistent association between anemia and LVH. Smaller studies with correction of severe anemia have demonstrated at least partial regression of LVH.

Other Effects of Anemia in Chronic Kidney Disease:

Anemia and its direct consequence, reduced oxygen carriage and delivery, may have other detrimental effects in patients with CKD. Worsening anemia could potentially accelerate the progression of kidney disease by depriving diseased kidneys of oxygen. A post hoc analysis of the RENAAL (Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan) trial was reported recently by Mohanram and associates. Among 1513 subjects with type 2 diabetes mellitus, initial Hb was an important predictor of renal outcome, including time to ESRD or doubling of serum creatinine. The risk was increased by 11% for every

1g/dL decrease in Hb concentration. For every 1 g/dL decrease in Hb, there was a 30% increase in infection risk.

A number of studies have assessed the effects of anemia on brain and cognitive function. The results have consistently linked anemia to impaired function and rHuEPO treatment to measurable improvements. (Benz RL et al) [17, 18]

BENEFICIAL EFFECTS OF CORRECTION OF ANEMIA:

1) Lesser need of blood transfusion
   - Lesser risk of Human Immunodeficiency Virus/Hepatitis C Virus
   - Less chances of alloantibodies (transplant rejection)
   - Less chances of iron overload
2) Improved quality of life & work tolerance
3) Regression of Left ventricular hypertrophy and infrequent Congestive heart failure
4) Reduced occurrences of angina / Myocardial infarction [11] (Anwer et al)

DIAGNOSTIC EVALUATION OF ANEMIA IN CKD:

Because the diagnosis of erythropoietin deficiency is one of exclusion, the evaluation should focus on excluding other causes of anemia with an appropriate history, examination and laboratory testing.

1) Haemoglobin–severity of anemia is assessed by measuring Hb% concentration, and haematocrit.

2) Complete blood count should be reviewed for any related problems in the leukocyte or platelet cell lines.
3) Red blood indices should be examined and anemia classified as microcytic, normocytic or Macrocytic.

Anemia of CKD usually results in a normocytic erythrocyte classification. If microcytosis is present, then iron deficiency, thalassemia and myelodysplasia should be considered. With macrocytosis, folic acid and vitamin B12 deficiency must be excluded. Echinocytes or burr cells were thought to be characteristic of chronic renal failure. However, even normal cells undergo a reversible transformation to burr cell-like echinocytes when exposed to a glass surface or incubated uremic plasma.

4) Faecal blood testing[19] (Bini EJ et al) and upper Gastrointestinal scopy should be performed to evaluate for occult gastrointestinal bleeding and motion examination for parasitic infestation should be done.
5) Iron profile including serum ferritin and transferrin saturation, serum transferrin receptors should be done to rule out iron deficient state. IRON

DEFICIENCY IN CKD:

The peripheral blood picture characterised by microcytosis and hypochromia, manifested by decreased RBC count, MCV, MCH, MCHC indicating reduced Hb due to iron deficiency, the degree of which depends upon the extent of iron deficiency which increases as CKD progresses.

DIAGNOSIS OF HYPOPROLIFERATIVE ANEMIA [4]

TESTS	IRON DEFICIENCY ANEMIA	INFLAMMATION	RENAL DISEASE
Anemia	Mild to severe	Mild	Mild to severe
MCV(fL)	60-90	80-90	90
MORPHOLOGY	Normo-microcytic	Normocytic	Normocytic
SERUM IRON(µg/L)	< 30	< 50	Normal
TIBC (µg/L)	> 360	<300	Normal
SATURATION %	<10	10-20	Normal
FERRITIN(µg/L)	< 15	30-200	115-150

ERYTHROPOIETIN [20]:

Erythropoietin (EPO) is a 34-kDa glycoprotein hematopoietic growth factor that can control the rate of red cell production by acting on erythroid precursors in the bone marrow production. Plasma EPO level is 4-27 U/L. In the kidney the peritubular interstitial cells outside the tubular basement membrane produce EPO. In patients with renal disease, the reduction in EPO production is roughly proportional to the degree of excretory impairment. [20](Erslev AJ)

Inflammatory cytokines in anemia[21, 22]:

Increased levels of inflammatory cytokines are detected in CKD. These cytokines inhibit the production of EPO and render erythroid cell insensitive to the action of EPO, leading to normocytic normochromic anemia. [21](Rogers JT et al)

Markers of Iron Status in CKD

The most routinely used iron markers in patients with CKD include serum Iron, transferrin saturation ratio; and serum ferritin. Although the bone marrow iron staining is the reference standard, it is a semi quantitative measure and rarely is used beyond investigational purposes [23, 24]. (Kalantar-zadheh K et al). Similarly, direct liver iron store assessment requires the invasive procedure of liver biopsy, although the indirect assessment *via* the superconducting quantum interference device (SQUID) may be a promising method that currently can be accessed only in very few centres.

Other non traditional used iron markers in patients with CKD, Include:

1. Reticulocyte hemoglobin content (CHr), is the amount of Hb present in each reticulocyte. CHr less than 28 pg indicate iron deficiency.

2. **Percentage of hypochromic red cells**[25] (Bovy C et al) is an indicator of iron deficiency as newly formed RBCs become hypochromic

as a consequence of iron deficiency. Hypochromic cells more than 10 %indicates iron deficiency.

3. **Soluble transferrin receptor concentration** , Because erythroid cells have the highest numbers of transferrin receptors of any cell in the body, and because transferrin receptor protein (TRP) is released by cells into the circulation, serum levels of TRP reflect the total erythroid marrow mass. TRP levels are elevated is absolute iron deficiency. Normal values are 4–9 g/L determined by immunoassay. This laboratory test is becoming increasingly available and, along with the serum ferritin, has been proposed to distinguish between iron deficiency and the anemia of chronic inflammation [4].

4. **Erythrocyte zinc protoporphyrin** (Canaves C et al) [26] increased levels reflect an inadequate iron supply to erythroid precursors to support hemoglobin synthesis. Normal values are <30 g/dL of red cells. In iron deficiency, values in excess of 100 g/dL are seen. The most common causes of increased red cell protoporphyrin levels are absolute or relative iron deficiency and lead poisoning.

ROLE OF HEPCIDIN:

Hepcidin, an acute phase reactant protein produced in the liver. Hepcidin inhibits intestinal iron absorption and iron release from macrophages and hepatocytes. Because hepcidin production is increased

by inflammation, and high hepcidin concentrations limit iron availability for erythropoiesis, hepcidin likely plays a major role in the anemia of inflammation and rHuEPO resistance.

If storage iron is elevated, then the liver synthesizes hepcidin, which feeds back to the gastrointestinal tract and to the placenta in pregnant women, preventing additional exogenous iron absorption. Hepcidin also inhibits the release of iron from the RE system to circulating transferrin.

Hepcidin activity in normal individuals is increased in the setting of inflammation/infection, primarily through the release of IL-6 by Kupffer cells in the liver. This explains the phenomenon of Reticuloendothelial blockade in which storage iron is not released to circulating transferrin, resulting in a high serum ferritin and low TSAT level. Not surprising, there is a significant correlation between hepcidin and serum ferritin because both are acute-phase reactants. [27](kalantar- zadeh et al)

Ferritin Synthesis: The Role of Inflammation[27]

Under normal amounts of body iron loading, most cells contain little ferritin, whereas cells in the reticuloendothelial system (RES) may contain larger amounts of ferritin. During the acute-phase response, pro-inflammatory cytokines such as IL-1 and TNF-alpha increase the synthesis of ferritin.

Hypothetically, higher amounts of ferritin may trap more body iron and protect the individual against worsening infection, the start of which invariably is associated with inflammation. Hence, inflammation-induced hyperferritinemia may result in a so-called "functional iron deficiency," which may be useful in "acute" inflammation by iron containment in the RES sites but harmful under "chronic" inflammation by leading to refractory anemia such as in CKD or other chronic disease states.

Hence, a low ferritin level (*e.g.*, 200 ng/ml in hemodialysis patients or 100ng/ml in nondialyzed patients with CKD) is a reliable indicator of iron deficiency, whereas a normal to moderately high serum ferritin does not rule out iron deficiency or indicate adequate or too much Fe on board.[27]

HYPERFERRITINEMIA IN CKD:

The increase in serum ferritin during inflammation, infection, liver disease, malignancies, and other non–iron-related conditions may hinder the ability to assess the iron status in CKD under the concurrent presence of foregoing conditions. Serum ferritin is a marker of malignancy, such as in neuroblastoma, renal cell carcinoma, or Hodgkin's lymphoma. Hyperferritinemia also is associated with liver dysfunction, probably because liver is the main organ to clear circulating ferritin molecules. High

ferritin levels have been reported in patients who had CKD with glomerular disease and proteinuria.

Chronic inflammation is common in patients with CKD, and up to 40 to 70% of patients with CKD may have increased C-reactive protein (CRP) levels on a chronic basis. Hence, inflammation probably is the most common confounder in CKD-associated hyperferritinemia and may contribute to it more strongly than Iron. There are many other, similar models of hyperferritinemia in chronic disease states, including rheumatoid arthritis, in which Iron deficiency is present in 50% of patients yet serum ferritin levels are normal or increased [27].

In patients with CKD, hyperferritinemia is paradoxically associated with Erythropoiesis stimulating agents hypo responsiveness and a more severe anemia. A significant association between serum CRP and ferritin that was independent of age, gender, race, and diabetes was found. Multivariate models showed that both CRP and TSAT, independent of each other, correlated significantly with serum ferritin. These findings suggest that a moderately high serum ferritin is not just a mere marker of Fe stores but more an indicator of inflammation and/or malnutrition as well as other non–iron related conditions in patients with CKD .[27]

SERUM FERRITIN AND MORTALITY IN CKD:

Hyperferritinemia-associated morbidity might be due to non–Iron-related factors. Because serum ferritin is a positive acute-phase reactant, hyperferritinemia associated increased risk for infection and death may be a mere epiphenomenon. Therefore, considering high ferritin levels as the primary cause of increased mortality in the setting of inflammation or infection and preventing optimal anemia management with intravenous Iron for serum ferritin levels 500 or 800 ng/ml may be irrational.[27]

IRON MONITORING [2, 8]

The K/DOQI anemia guidelines recommend that during the initiation of rHuEPO treatment, iron status be tested every month in patients not receiving ongoing iron repletion. Once rHuEPO dosing and iron maintenance have stabilized, the guidelines recommend monitoring at least every 3 months.

Serum ferritin is an indirect measure of storage iron[29]. The diagnostic value of serum ferritin, however, is limited by its behaviour as a potent acute-phase reactant. Clinical settings may arise in which ferritin values may be quite high even in the presence of iron deficiency, such as in hemodialysis patients, in whom the test probably has a sensitivity of only 41% to 54%. Because of the extraordinarily high rate of false negative

results, iron deficiency in hemodialysis patients cannot be excluded by serum ferritin more than 100ng/ml.

Percent transferrin saturation (TSAT) assesses the availability of circulating iron, calculated as TSAT = (serum iron/total iron-binding capacity) × 100. K/DOQI guidelines recommend using a value of less than 20% as an indicator of iron deficiency in patients with kidney disease.

Percentage of hypochromic red cells has been found to be a useful measure of iron status in CKD patients. The test has one important limitation: it is affected by changes in erythrocyte mean corpuscular volume (MCV). When samples are stored or shipped, the MCV may be significantly altered.

Reticulocyte hemoglobin content (CHr) is a direct measure of iron status at the level of the reticulocyte. Because it is a measure of content instead of concentration, it is unaffected by changes in cell volume. In addition, because reticulocytes circulate for only approximately 24 hours, test results can indicate very acute changes in iron status .Generally, a CHr value of less than 29 to 31pg indicates a need for more intensive iron treatment.

Hsu *et al.* [30] studied iron status in CKD in the NHANES III survey (1988 to 1994) and found iron indices suggestive of iron deficiency to be present and to contribute to anemia in many subjects.

Typical markers of iron deficiency used in CKD are serum ferritin <100 ng/ml and TSAT < 20%. Clinicians often use these thresholds to base iron treatment decisions, and K/DOQI guidelines recommend these levels in nondialysis CKD. Specifically, the K/DOQI guidelines indicate that if either value is low then iron treatment is recommended.

Steven Fishbane et al [31] primary finding is that between 57.8 and 72.8% of subjects with CKD have either serum ferritin < 100 ng/ml or TSAT < 20%. In contrast to these relatively high values of serum ferritin (100 ng/ml) and TSAT (20%) that indicate insufficient iron in CKD, in the general population lower thresholds of serum ferritin (15 to 30 ng/ml) and TSAT (15%) are often used. The great prevalence of low iron indices found may not indicate iron deficiency *per se*, but rather impaired iron delivery concurrent with inflammation, a complex syndrome that occurs with progressive CKD. However, it is plausible that iron deficiency might be more common than expected because in CKD the prevalence of gastrointestinal pathology with blood loss is probably increased, sampling of blood for laboratory testing is common, and hospitalizations and surgery for other intercurrent illness could contribute to blood loss. In addition, many patients are treated with Erythropoiesis stimulating agents, further depleting iron stores.

Gotloib *et al.* [32] these investigators performed sternal bone marrow biopsies on 47 patients with CKD and Hb < 12 g/dl. Remarkably, severe iron deficiency was found in 46 of 47 subjects. Patients were subsequently treated with intravenous iron, with most responding with improved Hb concentration [32], indicating that iron deficiency is a common problem among patients with nondialysis CKD.

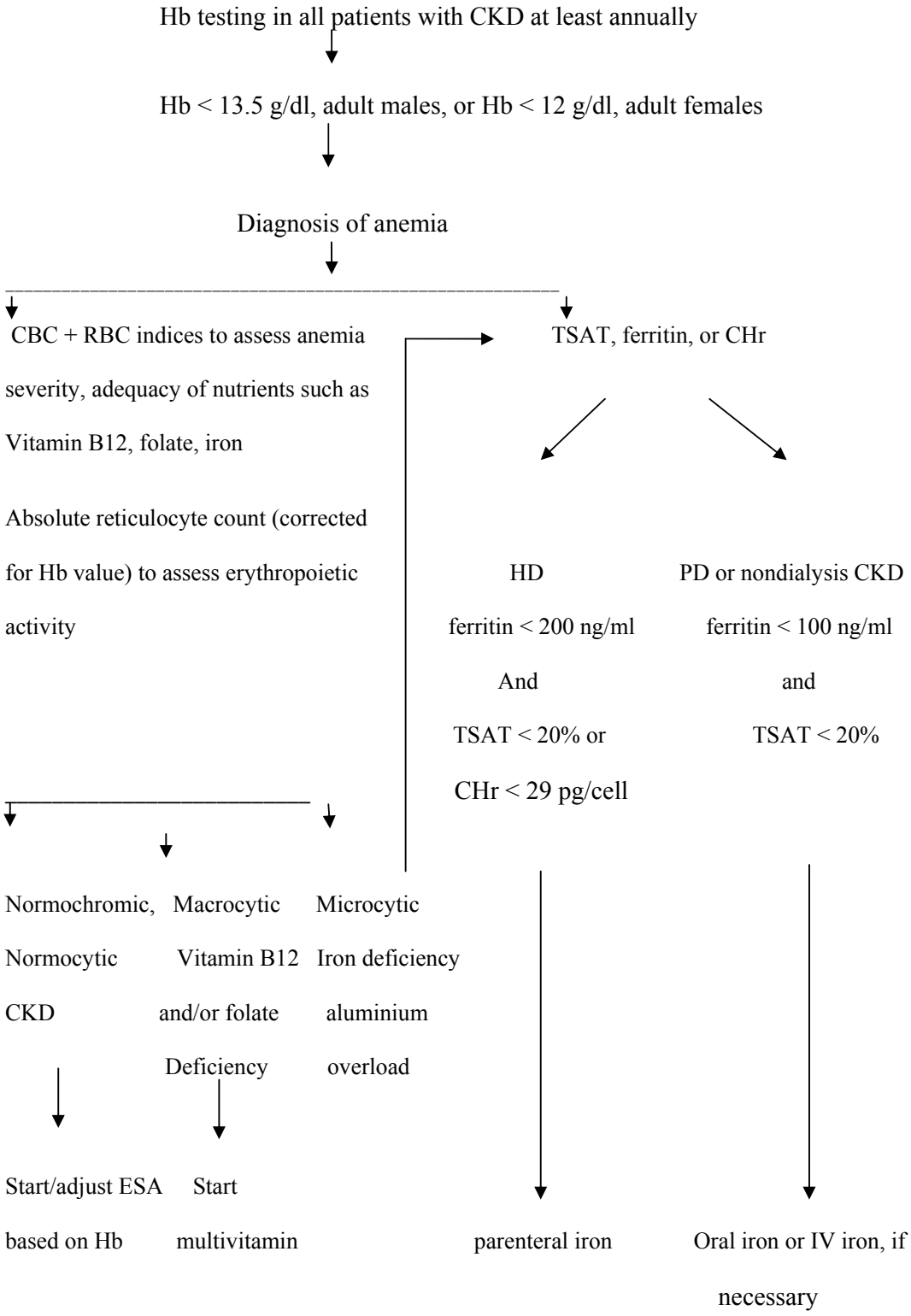
Evaluation of Iron Storage-Serum Ferritin

The most recent K/DOQI guidelines recently have recommended that serum ferritin should be maintained greater than 200ng/mL for haemodialysis' patients. For patients with CKD not yet on dialysis or those on peritoneal dialysis, The K/DOQI guidelines recommend maintaining serum ferritin greater than 100ng/mL in these populations.

Evaluation of Iron Availability—Transferrin Saturation and Reticulocyte Hemoglobin Content (CHr)

The K/DOQI recommended level of transferrin saturation is 20%, for all populations with CKD. The K/DOQI recommendation for reticulocyte haemoglobin (CHr) content is 29pg.

Anemia treatment algorithm



TREATMENT OF ANEMIA: [8, 2]

1. Dialysis

Dialysis per se typically has little effect with regard to correcting the anemia, although a mild increase in hemoglobin concentration may result from the decrease in bleeding tendency.

2. Iron and Folate Supplementation

The goals of iron therapy are:

To achieve and maintain a target range Hb level,

To avoid depletion of storage iron,

To prevent iron deficient erythropoiesis,

To minimise the dose of ESA

3. Transfusion Therapy

Transfusions with packed red cells are necessary to counteract the effects of acute blood loss. Transfusions occasionally are needed to maintain acceptable hemoglobin concentrations in patients who do not respond adequately to EPO.

4. Recombinant Erythropoietin Administration:

Currently available Erythropoiesis stimulating agents are[11]

1. Short acting: Epoietin alpha, Epoietin beta
2. Long acting: Darbepoietin alpha

3. Newer erythropoiesis stimulation therapies: Continuous erythropoietin receptor activator (C.E.R.A), peptide based ESA hematide, Synthetic eryhropoiesis protein (SEP), EPO gene therapy.

The National Kidney Foundation has published detailed guidelines for EPO administration to patients with the anemia of chronic renal diseases. In short, the presence of an anemia with hematocrit of less than 33 percent or hemoglobin of less than 11 gm/dl should initiate a thorough search for conditions unrelated to decreased EPO production or action. Measurements of folic acid and B_{12} levels should be carried out, with special attention to iron, iron-binding capacity, and ferritin levels. Determination of EPO levels is not necessary.

The National Kidney Foundation recommended an increase in the target hematocrit to 33 to 36 percent and target hemoglobin to 11 to 12gm/dl. To achieve the target hematocrit within 3 to 4 months of therapy, the initial EPO dose in adult patients should be 80 to 120 units/kg/week divided into two or three subcutaneous injections or 120 to 180units/kg/week given as three intravenous injections. The response should be monitored by measuring hematocrit and haemoglobin at least once every 2 weeks. Once the target hematocrit is reached, most adult patients can be maintained by a total EPO administration of approximately 50to100units/kg/week.

Adequate iron supplies must be maintained for erythropoiesis. A diagnosis of absolute or functional iron deficiency should be made before patients are supplemented with IV iron. The most widely used criteria include a ferritin level less than 100 ng/ml and/or transferrin saturation less than 20 percent. [29] (Jaime Caro et al)

The most widely used IV iron preparations are iron-dextran, iron-sucrose, and iron-gluconate. Iron-dextran and iron-sucrose can induce anaphylactic reactions; iron-gluconate can induce hypotension.

Causes of erythropoietin resistance:

Common causes:

The most common causes of a poor response are

Inadequate iron supply,

Persistent iron deficiency,

Hospitalisation for infection,

Temporary and permanent catheter insertion,

Hypoalbuminemia,

Elevated CRP level.

Aluminium toxicity may be responsible for resistance to treatment and should be suspected in patients with microcytic red cell indices.

Uncommon causes: pancytopenia, aplastic anemia, haemolytic anemia, chronic blood loss, inflammatory diseases, infection, ACE inhibitors.[11]

Adverse Effects of Erythropoietin

Hypertension- most common complication

Seizures,

Thrombosis of arteriovenous fistulas,

High potassium levels

Hyperphosphatemia.

Pure red cell aplasia (PRCA). Patients with PRCA present with a low absolute reticulocyte count and resistance to EPO treatment. Marrow examinations have shown a decrease in erythroid precursors.

Adjuvant to erythropoietin, Erythropoiesis stimulating agents (ESA) or iron therapy: [11] the aim of add on therapy are to enhance responsiveness to ESA hypo responsive patients and to decrease cost by decreasing ESA doses ; L-carnitine, vitamin E, androgens, statins , vitamin C. However KF-K/DOQI found inefficient evidence to recommends use of adjuvant in management of anemia in patients with CKD.

Amelioration of the anemia has resulted in a variety of beneficial effects and in general has dramatically improved the quality of life of uremic patients.

DISORDERS OF HEMOSTASIS IN CHRONIC KIDNEY DISEASE[2, 4, 28, 29]

Excessive bleeding has long been recognized as an important complication of the uremic state. That includes epistaxis, gastrointestinal haemorrhage, excessive bleeding with tooth brushing, or easy bruisability. More severe bleeding episodes tend to occur with trauma or after invasive procedures, such as renal biopsy, rather than spontaneously. Hemopericardium and subcapsular hematoma of liver occur but less frequently than other bleeding manifestations.

It has long been noted that bleeding in uremic patients occurs despite normal or elevated circulating levels of coagulation factors. Whereas the number of circulating platelets is generally normal, the function of platelets is often impaired. [6] (Escolar et al)

Other coagulation parameters (partial thromboplastin time, prothrombin time, and fibrinogen) are not altered in uremia.

Evidence for platelet dysfunction includes elevated bleeding time, diminished in vitro response to adenosine diphosphate and epinephrine, and reduced ristocetin-induced platelet agglutination.[5] (Fegurson HJ et al)

The most consistent abnormality in platelet function in uremia is impaired interaction of platelets with the vascular sub endothelium. As a result, platelet adhesion and aggregation are hindered. The best measure of

platelet- vessel wall interaction is the bleeding time, a simple method tested by making small incision of the skin and measuring the time from first drop of blood to the last oozing of blood from the cut.

The platelet functional abnormalities are:

- Abnormal aggregation to ADP, adrenaline, collagen
- Decreased platelet adhesiveness
- Reduced platelet factor 3 availability
- Acquired storage pool defect
- Abnormal prostaglandin metabolism
- Increased prostacyclin
- Defective platelet cyclooxygenase

Platelet receptors that play a critical role in adhesion to the vessel wall and aggregation, GP1b and GPIIb-IIIa, are probably not significantly reduced in quantity in uremia. However, interaction of these receptors with vessel wall proteins may be abnormal. In particular, activation of GPIIb-IIIa to facilitate its adhesion to vWF may be impaired. Finally, the platelet cytoskeleton may be altered, with diminished actin incorporation and suboptimal intracellular trafficking of molecules.[2]

ROLE OF ANEMIA IN PLATELET DYSFUNCTION:

Anemia is an important contributor to uremic platelet dysfunction. During normal circulation, erythrocytes tend to force the flow of platelets

radially, away from the centre of flow and toward the endothelial surfaces. When vascular injury occurs, platelets are in closer opposition to the vessel wall, facilitating platelet adherence and activation by vessel wall constituents such as collagen. With anemia, more platelets circulate in the centre of the vessel, further from endothelial surfaces, hindering efficient platelet activation. In addition, anemia may contribute to platelet dysfunction because adenosine diphosphate release by erythrocytes normally stimulates platelet interaction with collagen.[2]

THERAPY FOR BLEEDING IN UREMIC PATIENTS: [2]

1. **Dialysis:**

Dialysis reduces uremic platelet dysfunction and the risk for bleeding.

2**. Correction of anemia:**

Treatment of anemia may help reverse platelet dysfunction, as both transfusion of blood and rHuEPO therapy have been found to be beneficial.

3**. Cryoprecipitate and Desmopressin:**

Desmopressin (DDAVP) is often used to treat uremic bleeding.

Other treatments for uremic bleeding include infusion of cryoprecipitate, a plasma product rich in vWF and fibrinogen. Estrogens also improve platelet function by action of inhibition of vascular nitric oxide production.

LEUKOCYTES IN CKD: [29]

The total and differential leukocyte count and the platelet count usually are normal, but, as with all other hematologic parameters, the underlying disorder plays a modifying role. Uremia and dialysis may have an effect on leukocytes and platelets. The phagocytic activity of granulocytes may be reduced, and complement activation by the hemodialysis membrane may cause pulmonary leukostasis with temporary granulocytopenia. Cell-mediated immunity is depressed, resulting in an increased incidence of infections but also prolonged graft survival. Granulocytes show decreased migration and abnormal chemotactic activity.[29] (Jaime Caro et al)

MATERIALS AND METHODS

Materials:

This study was conducted at Government Rajaji Hospital during the period of April 2011 to October 2011. Fifty four non dialysis chronic kidney disease patients undergoing conservative management in medicine/nephrology units were enrolled into the study. The study subjects were newly diagnosed chronic kidney disease patients of either sex. Healthy adult individuals were recruited as controls. To ensure homogeneity between the control and CKD population, healthy individuals were selected from the friends and relatives accompanying the CKD patients.

Haematological profile was done in Pathology Department and renal parameters, Serum Iron indices in subjects and controls were done at the Department of Biochemistry, Madurai Medical College.

The study was approved by the Ethical committee, Government Rajaji Hospital. An informed consent was obtained from all study participants.

Design: cross-sectional study

General physical examination, urinalysis and blood sugar, and creatinine estimation were done to establish the healthy nature of controls. A total of 54 patients and 20 controls were studied.

Period of study : April 2011 to October 2011.

Diagnostic criteria:

1. Bilateral contracted kidneys
2. GFR <60 mL/min/1.73m^2.

Exclusion criteria:

Conditions that may alter the iron profile and RBC morphology were excluded on the basis of detailed history and clinical examination and basic investigations.

They include:

1. Age less than 18 years
2. Evidence of acute infection or trauma in the last four weeks
3. History of parenteral iron injection in the last 14 days
4. History of blood transfusion in the last one month
5. Hemoglobinopathies
6. Malignancy
7. Recent overt blood loss
8. On dialysis, Post-transplant status
9. Chronic infections like tuberculosis
10. Bleeding disorders, Previously diagnosed anemia and treated
11. Nephrotic syndrome
12. Chronic liver disease

13. HIV infection
14. Malabsorption
15. Steroid therapy
16. Patients receiving EPO therapy.

Methods:

Clinical examination included:

1. Weight, height, vital parameters
2. Major systems examination

Haematological investigations:

Haemoglobin, Red blood cell count, White blood cell count, Haematocrit, Differential count, MCV, MCH, MCHC, Platelet count, RDW, Peripheral smear, Bleeding time Clotting time, ESR, Serum ferritin, Total iron binding capacity, Serum iron were done.

Automated hemogram was done.

Peripheral smear was done. ESR was measured by Wintrobes method.

Biochemical investigations:

Blood sugar, renal parameters (blood urea, serum creatinine), serum electrolytes, Urine spot protein creatinine ratio.

Serum ferritin: The serum ferritin was determined by enzyme linked immunosorbent assay. Iron level was determined by Ferrozine method

without deproteinization. Total iron binding capacity (TIBC) was determined by Spectrophotometric Assay.

Transferrin saturation calculated by using formula (TSAT);

$$TSAT = (\text{serum iron}/\text{total iron-binding capacity}) \times 100.$$

Ethical committee approval: Obtained

Consent : Informed consent was obtained

Financial support : Nil

Conflict of interest : Nil

Statistical Tools

The information collected regarding all the selected cases were recorded in a Master Chart. Data analysis was done with the help of computer using **Epidemiological Information Package (EPI 2010)** developed by Centre for Disease Control, Atlanta.

Using this software range, frequencies, percentages, means, standard deviations, chi square and 'p' values were calculated. Kruskul Wallis chi-square test was used to test the significance of difference between quantitative variables and Yate's chi square test for qualitative variables. A 'p' value less than 0.05 is taken to denote significant relationship.

RESULTS AND ANALYSIS OF OBSERVED DATA

Table 1: Age distribution

Age group	Study cases (CKD)		Control Cases (Normal)	
	No	**%**	**No**	**%**
<20 years	2	3.7	1	5
21-30 years	8	14.8	3	15
31-40 years	12	22.2	6	30
41-50 years	15	27.8	5	25
50-60 years	17	31.5	5	25
Total	54	100	20	100
Range	19-60		20-58	
Mean	43.3		40.7	
SD	11.8		11.6	

The study group had an age of 43.3 $\pm$11.8 years and the control group an age of 40.7 $\pm$11.6 years.

MEAN AGE

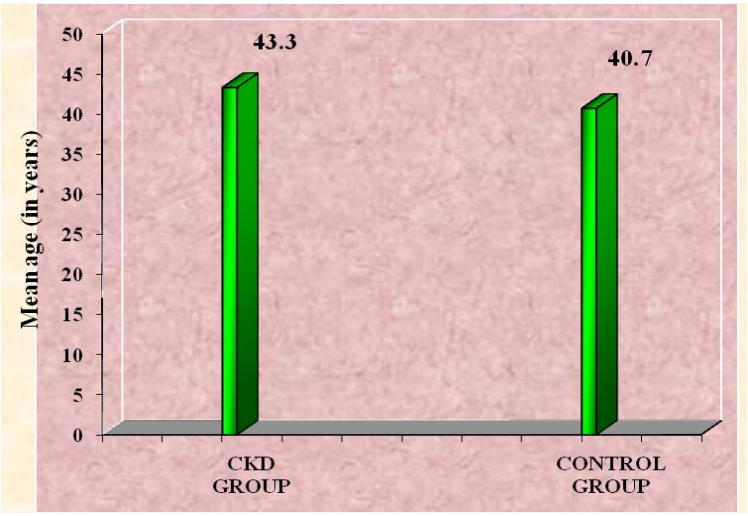

Mean age (in years)
50
45
40
35
30
25
20
15
10
5
0
43.3
40.7
CKD GROUP
CONTROL GROUP

Table 2: Sex distribution

Sex	Study cases		Control Cases	
	No	%	No	%
Male	42	77.8	15	75
Female	12	22.2	5	25
Total	54	100	20	100

In this study out of 54 cases 42 are males and 12 are females. Males are 77.8% and females are 22.2%.

SEX DISTRIBUTION

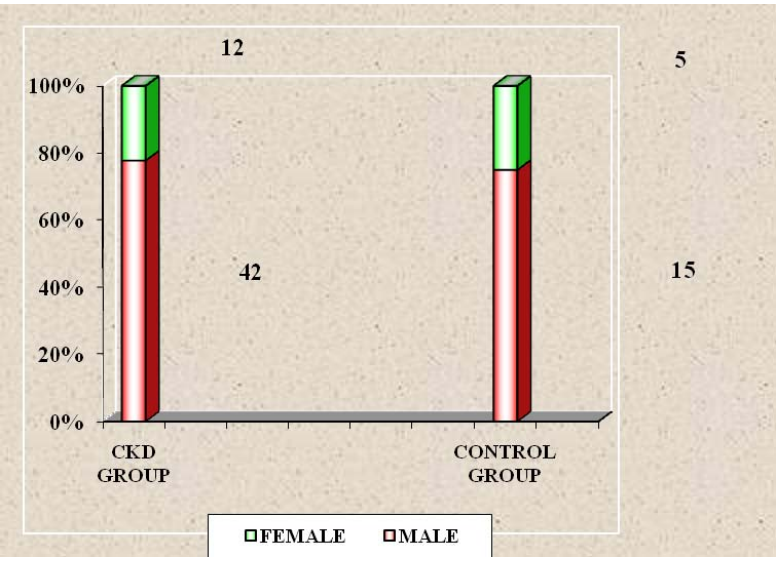


Table 3: Duration of illness

Parameter	Duration of CKD in months
Range	3-24
Mean	8.57
SD	4.2

Mean duration of illness ranged from 3 months to 24 months with an average of 8.57 months.

Table 4: Glomerular filtration Rate (GFR)/ CKD stages

CKD stages/GFR ($ml/min/m^2$)	Cases	
	No	**%**
Stage 5 (< 15)	23	42.6
Stage 4 (15 -29)	19	35.2
Stage 3 (30-59)	12	22.2
Stage 2 (60-89)	-	-
Stage 1 (≥ 90)	-	-
Total	54	100
Range	2.1 – 47.8	
Mean	18.77	
SD	12.01	

Among 54 CKD cases, Glomerular Filtration Rate (GFR) ranged from 2.1 to 47.8 $ml/min/m^2$. The mean GFR was 18.77 $ml/min/m^2$ and SD 12.01. There were no cases of stage 1 and 2 in the selected cases.

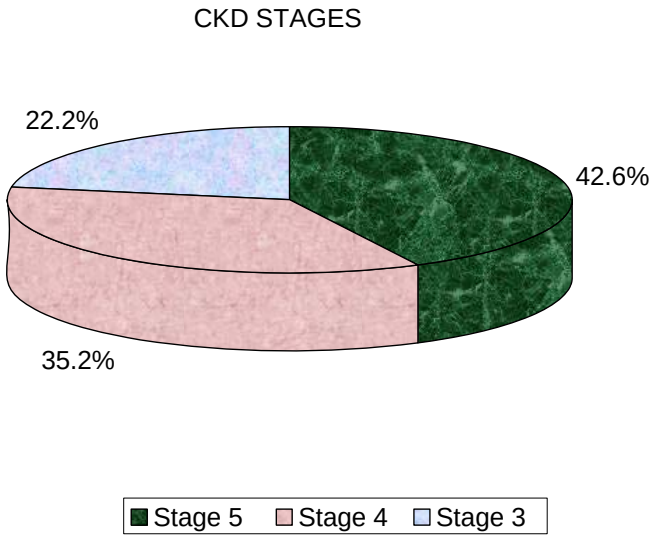

CKD STAGES
22.2%
42.6%
35.2%
Stage 5
Stage 4
Stage 3

Table 5: Risk factors for CKD

Risk factor	No. of cases			
	Present		Absent	
	No	%	No	%
Diabetes mellitus	11	20.4	43	79.6
Hypertension	27	50	27	50

Hypertension was present in 50% and Diabetes Mellitus in 20.4% of study cases.

Table 6: Other risk factors for Anemia:

Other risk factors for anemia	No of cases
History of bleeding manifestation	2
Motion ova & cyst present	2
Motion for occult blood	1

History of bleeding manifestation (gum bleeding, ecchymosis) was present in 2 cases, motion ova & cyst present in 2 cases and motion for occult blood was positive in one case.

Table 7: Haematological profile between study and control group

Variable	Study group		Control group		'p'
	Mean	**SD**	**Mean**	**SD**	
Hb (gm/dl)	8.01	1.78	13.34	0.85	**0.0001 Significant**
RBC count (million/cumm)	3.18	0.7	4.58	0.45	**0.0001 Significant**
PCV %	25.58	4.36	40.37	3.18	**0.0001 Significant**
Platelet count (lakhs/cumm)	3.33	0.69	2.94	0.77	0.0517 Not Significant
MCV (fL)	80.83	14.12	83.82	4.03	0.7104 Not significant
MCH (pg)	26.15	5.12	28.72	1.64	0.0775 Not significant
MCHC (g/dl)	31.38	4.06	33.0	1.01	0.2786 Not significant
RDW%	15.92	4.33	13.93	0.63	0.1902 Not significant
ESR (mm/hr)	26.81	16.5	15.65	3.08	**0.0056 Significant**

When analyzing above data between study and control group, there were statistically significant differences seen in Hemoglobin (Hb), RBC

count, PCV and ESR ($p<0.005$). Hb, RBC count PCV were low and ESR was high compared to control.

Mean Hemoglobin was 8.01gm/dl and mean haemoglobin in males was 8.03 gm/dl and in females mean haemoglobin 7.71 gm/dl. Mean RBC count was 3.18 million/mm^3, mean PCV 25.58%. Mean Red cell Distribution Width were15.92%. Erythrocyte Sedimentation Rate was elevated in 35 cases (64.8%).

Hemoglobin and Packed Cell Volume was low in all cases.

7 cases had neutrophilic leukocytosis and 3 cases had lymphocytosis. 6 patients had eosinophilia. No patient had features of lymphoma, leukaemia. 7 patients had thrombocytosis.

Table 8: Association between CKD stages and other quantitative Haematological parameters

Parameter	Values (Mean ±SD) in cases with			'p'
	CKD 3	**CKD4**	**CKD5**	
Hb(gm/dl)	9.23 ±1.36	8.32 ±1.7	7.0 ±1.54	**0.0002 Significant**
RBC (million/cumm)	3.45 ±0.53	3.22 ±0.72	3.01 ±0.73	0.0892 Not significant
PCV %	27.88 ±3.82	26.61 ±4.29	23.52 ±3.89	**0.0059 Significant**
MCV (fL)	81.5 ±8.9	82.4 ±9.3	81 ±11.8	0.9704 Not significant
MCH (pg)	27.4 ±5	25.7 ±5.7	25.9 ±4.8	0.5124 Not significant
MCHC (g/dL)	30.2 ±3.4	31.2 ±4.5	32.1 ±4.0	0.3517 Not significant
RDW %	16.1 ±4.6	16.5 ±5.5	15.3 ±3.1	0.7476 Not significant
Duration (in months)	6.25 ±3.28	8.47 ±3.92	9.87 ±4.44	**0.0264 Significant**

There were statistically significant associations between Hb% ($p= 0.0002$), PCV ($p<0.005$) and duration of illness and CKD stages. ($p< 0.05$). As the CKD stage increases, the level of hemoglobin, packed cell volume decreases. These relationships have got statistical significance.

Table 9: Bleeding time and clotting time

Parameter	Bleeding time in minutes		Clotting time in minutes	
	No	%	No	%
Normal	51	94.4	54	100
Increased	3	5.6	-	-
Total	54	100	54	100

Bleeding time increased in 3 patients (5.6%). Clotting time was normal in all patients.

Table 10: Peripheral smear

Peripheral smear	**Study cases**		**Control Cases**	
	No	**%**	**No**	**%**
Normocytic normochromic anemia	38	70.4	-	-
Microcytic hypochromic anaemia	11	20.4	-	-
Both types present	5	9.2	1	5
Normal	-	-	19	95
Total	54	100	20	100
'p'	**0.0001 Significant**			

All the patients in the study cases had anaemia whereas only 5% in the control group had it. This difference was statistically significant (p = 0.0001). When analyzing above data 38 patients (70.4%) had normocytic normochromic anemia, 11patients (20.4%) had microcytic hypochromic anemia and 5 patients (9.2%) had both type of morphology in peripheral smear picture.

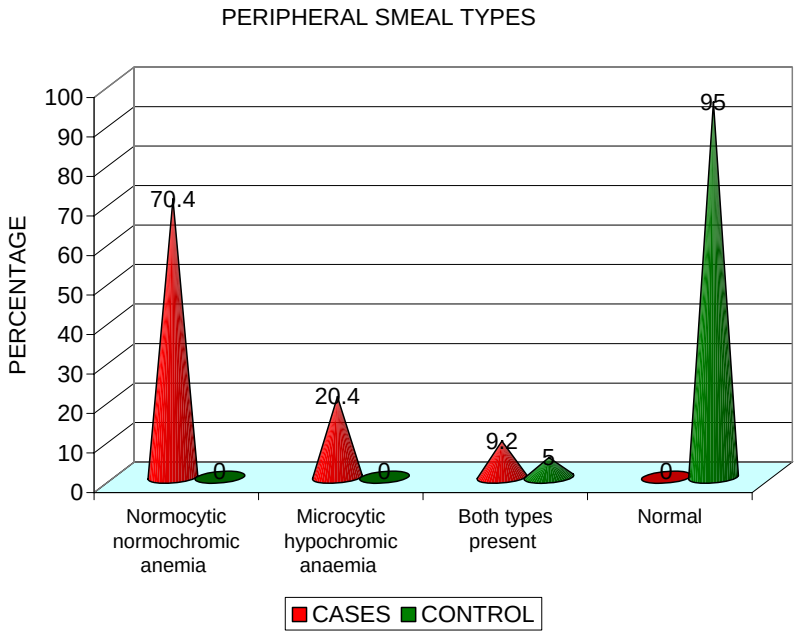

PERIPHERAL SMEAL TYPES
PERCENTAGE
100
90
80
70
60
50
40
30
20
10
0
70.4
0
20.4
0
9.2
5
0
95
Normocytic normochromic anemia
Microcytic hypochromic anaemia
Both types present
Normal
CASES
CONTROL

Table 11: Relationship between type of Peripheral smear and serum Ferritin (µg/l);

Transferrin saturation %(TSAT)

Peripheral Smear Type	No of cases	Mean TSAT	Mean FERRITIN
Normocytic normochromic	38	32.46	381.98
Microcytic hypochromic	11	12.61	26.58
Both	5	14.26	43.82

There was a statistically significant relationship between peripheral smear type and TSAT (p=<0.001) and also significant relationship between peripheral smear type and ferritin (p=<0.002). Serum ferritin level and transferrin saturation was low in patients with microcytic hypochromic anemia.

Relationship between type of Peripheral smear and serum Ferritin (µg/l); Transferrin saturation %(TSAT)

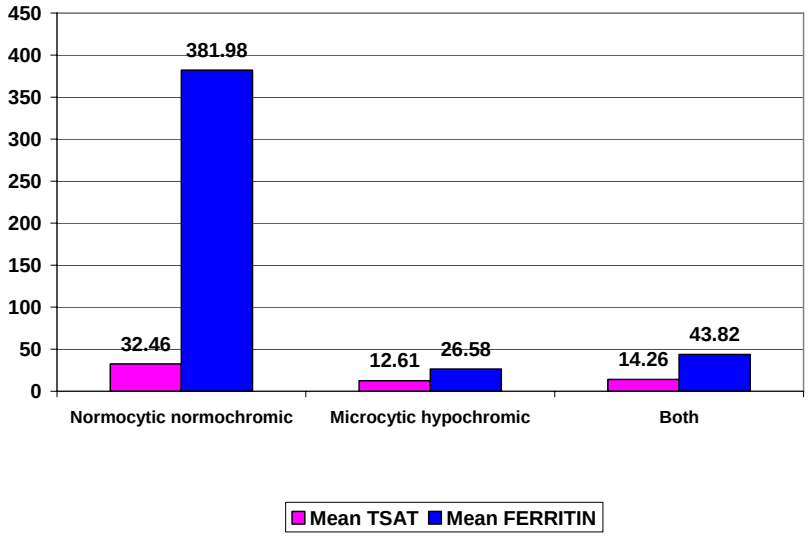


Table 12: Hemoglobin and Left ventricular hypertrophy (LVH)

LVH	No of cases	Mean Hb(gm/dl)	SD
Present	22	6.54	1.57
Absent	32	8.93	1.15

Left ventricular hypertrophy was present in 40.7% of CKD cases (22cases). There was statistically significant relationship between Hemoglobin level and LVH. ($P<0.001$)

Table 13: Serum Iron indices

Serum Iron Indices	Study group		Control group		'p'
	Mean	SD	Mean	SD	
Iron (µg/l)	68.7	31.9	82.7	25.4	0.0182 Significant
TIBC(µg/l)	287.4	77.7	300.2	45.5	0.7936 Not significant
TSAT %	26.74	16.5	28.8	10.09	0.2896 Not significant
Ferritin(µg/l)	278.3	340.4	109.4	62.0	0.0479 Significant

Among the serum iron indices between study and control cases, Iron and Ferritin values were significantly different, ($p < 0.05$). TIBC, TSAT values were not significantly different.

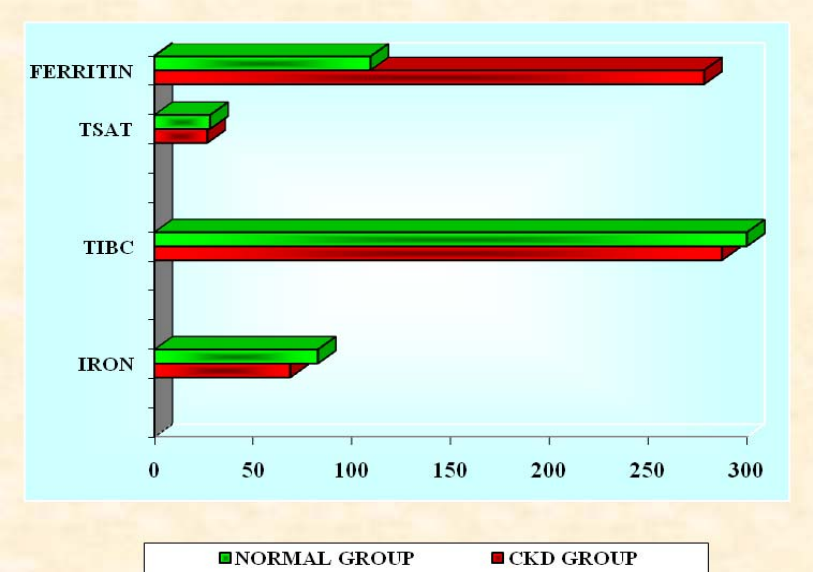


Table 14: Serum iron indices and CKD stage

CKD stage	Value (Mean ±SD) of			
	Iron(µg/l)	**TIBC**(µg/l)	**TSAT%**	**Ferritin**(µg/l)
3	70.7 ±32.8	269.9 ±76.2	31.6 ±23.5	357.9 ±519.8
4	69.3 ±39.4	300.3 ±77	25.3 ±16.7	254.8 ±254.5
5	67.2 ±25.2	286 ±80.5	25.4 ±11.6	256.1 ±294.4
'p'	0.7939 Not significant	0.4799 Not significant	0.7688 Not significant	0.9591 Not significant

Serum iron indices and severity of CKD did not have statistically significant relationship with serum iron profile.

Table 15: %TSAT and CKD Stage

CKD STAGE	TSAT %					
	< 20%		≥ 20%		Mean	SD
	No	%	No	%		
3 (12)	6	50	6	50	31.6	23.5
4 (19)	9	47.4	10	52.6	25.3	16.7
5 (23)	9	39.1	14	60.9	25.4	11.6
Total (54)	24	**44.4**	30	55.6	26.7	16.5
'p'	0.7688 Not significant					

Prevalence of Transferrin saturation (TSAT) < 20% is 44.4% of study cases. No significant relation between CKD stage and Transferrin saturation. Transferrin saturation (TSAT) >20% was present in 55.6% cases.

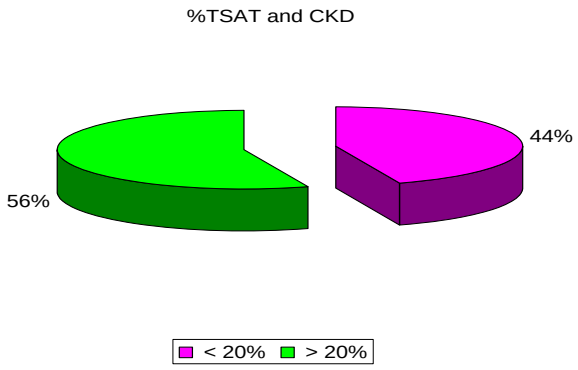


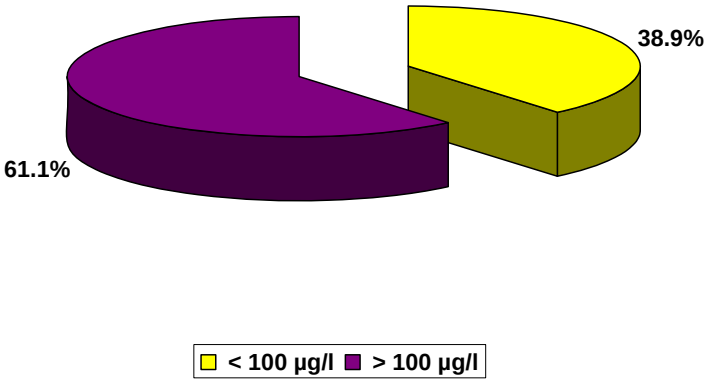
Table 16: Ferritin (µg/L) and CKD Stage

CKD STAGE	Ferritin					
	< 100microg/L		≥ 100microg/L		Mean	SD
	No	%	No	%		
3 (12)	5	41.7	7	58.3	357.9	519.8
4 (19)	9	47.4	10	52.6	254.8	254.5
5 (23)	7	30.4	16	69.6	256.1	294.4
Total (54)	21	**38.9**	33	61.1	278.3	340.4
'p'	0.9591 Not significant					

Prevalence of Serum ferritin level <100 micro gm/L is 38.9% of the study cases. No significant relation between CKD stage and serum ferritin. Serum ferritin level > 100 micro gm/L was present in 61.1% cases.

9 cases (16.67%) had serum ferritin >500 micro gm/l.

Ferritin (µg/L) and CKD



DISCUSSION

Chronic kidney disease is a major public health problem and major cause of morbidity and mortality worldwide. The actual prevalence of the initial stages of CKD is much more than the late stages.

However in clinical practice prevalence of stage 4 and 5 appears to be more because initial stages are asymptomatic and people present themselves when severity of symptoms increases.

Anemia of chronic kidney disease is multifactorial in origin. The renal community has long recognized that anemia can impair the quality of life of patients and lead to irreversible cardiac consequences[33, 34]. (Levy AS et al)

Anemia, an easily reversible feature of end-stage renal disease, is an independent risk factor for cardiac disease, as well as mortality in end stage renal disease patients [33, 34].

Available evidence demonstrates that: Both iron and erythropoietin are needed to produce red blood cells; as a result, unless adequate iron is available, Erythropoietin will be relatively ineffective. Although no tests are perfect indicators of the adequacy of iron stores, the TSAT and serum ferritin are the best measures of the body's iron status that we currently have[35, 36, 37]. Given the prevalence of iron deficiency in CKD patients, and the sensitivity and specificity of TSAT and serum ferritin in detection of

iron deficiency, the likelihood of iron deficiency is sufficiently high when TSAT is <20% and the serum ferritin is <100 ng/mL. Therefore, the TSAT and serum ferritin should be maintained at a level of >20% and >100 ng/mL, respectively, in all non dialysis chronic kidney disease patients.

This study was undertaken with the aim to study the haematological profile and identifying the prevalence of iron deficiency anemia (according to NKF- K/DOQI Guidelines) in non dialysis chronic kidney disease patients.

Among the 54 patients selected for the study the sex distribution was 42 male and 12 females and the mean age of the study group was 43.3yrs. In assessing the risk factors of chronic kidney disease in our patients, diabetes were prevalent in 20.4% meanwhile 50% of them had hypertension. Afshar et al[38] in their study found 49.1% patients were diabetic and 28.3% were hypertensive among CKD patients.

In our study mean duration of illness was 8.57 months. Applying the NKF staging of CKD, most of our patients came under stage 3, 4 or 5 who were awaiting some form of renal replacement therapy as the last treatment option. Mean Glomerular Filtration Rate was 18.77 ml/min/m^2.

At the time of presentation in our study, about 40.7% had left ventricular hypertrophy as per ECG criteria. Anemia and hypertension are the most important causes of left ventricular hypertrophy in chronic kidney

disease patients. Hypertension and left ventricular hypertrophy are the major risk factors for cardiovascular death in patients with CKD.

The relationship between anemia and cardiac disease in CKD was studied by Levin and co-workers, [16, 33] in their study Echocardiograms were performed in 175 patients attending a renal insufficiency clinic. LVH was found to be present in 38.9% of patients. The prevalence of LVH progressively increased with declining levels of renal function; 26.7% of patients with creatinine clearance (CrCl) greater than 50 mL/min, 30.8% with CrCl of 25 to 49 mL/min, and 45.2% with CrCl less than 25 mL/min. Each 1 g/dL decrease in Hb was associated with a 6% increase in risk for LVH. Furthermore, these investigators performed two echocardiograms 1 year apart on 246 patients with early stages of CKD to determine factors responsible for subsequent worsening of LVH. Worsening anemia proved to be an important predictor, with Hb decreasing 0.85 g/dL in patients with ventricular growth compared with a decrease of 0.11 g/dL among patients with stable LVH.

Among study and control group there was a statistically significant difference present between Hemoglobin, Red Blood Cell count, Packed Cell Volume, and Erythrocyte Sedimentation Rate.

Anemia was universal in our study and it showed direct linear relationship with reduction in the GFR. The mean Hemoglobin in our

patients was 8.01 gm/dl. There was a significant reduction in Packed Cell Volume with progressive increase in stage of Chronic Kidney Disease. Afshar et al[38] and Khanam et al[39] also found same linear relationship between Hb and GFR. Khanam et al[39] also found reduction in PCV with decreasing GFR. Ijoma et al[47] found mean haemoglobin 10.57 gm/dl in stage 3, 8.84 gm/dl in stage 4, 7.33 gm/dl in stage 5 chronic kidney disease. This states that anemia is very well correlated with severity of chronic kidney disease

The lower GFR or EPO production, greater loss of haematopoietic elements and inflammation can lead to lower haemoglobin and hematocrit level in CKD patients. As anorexia, nausea, vomiting are the common features of CKD patients, less dietary intake of nutrients needed for erythropoiesis might also be a factor for anemia. In developing countries like India, parasitic infestation, low socioeconomic status may play a role in nutritional deficiency and anemia. More over CKD patients are on protein restricted diet which might also have some role for occurrence of anemia in these patients.[39]

Peripheral smear was done in our patients with the aim to classify the type of anemia. As the conventionally taught normocytic normochromic anemia was found in 70.4 % of the patients and microcytic hypochromic anemia in another 20.4%, and 9.2% had both type of morphology in

peripheral smear picture. Afsar et al [38]also found normocytic nomochromic anemia in 80% patients and microcytic hypochromic anemia in 15% patients. Talwar et al[40] found 60% patients with microcytic hypochromic anemia, 30% patients normocytic normochromic anemia in their study.

MCV, MCH, MCHC of the patients with normocytic normochromic anemia were normal and low in hypochromic microcytic anemia. There was a statistically significant relationship between peripheral smear type and TSAT and also between the peripheral smear type and ferritin. Serum ferritin level and transferrin saturation was low in microcytic hypochromic anemia.

Increased bleeding time was present in 3 cases (5.6%). Akisola et al[41] reported increased bleeding time 25.6% in their study. So increased bleeding time in some of the patient calls for caution in surgical procedure in CKD patients and correction of anemia may improve the bleeding time abnormality.

In our study 7 cases had neutrophilic leukocytosis, 3 cases had lymphocytosis and 6 cases had eosinophilia. No patients had leukaemia or lymphoma. Talwar et al[40] found in their study found increased leukocyte and eosinophil count in 32% patients. The presence of uremic toxins itself can lead to such changes, in addition to the presence of infection.

A raised ESR in 64.8% patients may be due to presence of low grade chronic inflammation and anemia in Chronic Kidney Disease. Afshar et al[38] found elevated ESR in more than half of patients in their study.

There has long been a great interest in iron tests and iron status in hemodialysis patients[42, 43]. In contrast, far less is known regarding iron status of patients with nondialysis Chronic Kidney Disease. It should be noted that our results are most applicable to patients with estimated GFR <60 ml/min in which CKD is most likely to be present. Hsu et al.[30] studied iron status in CKD in the NHANES III survey (1988 to 1994) and found iron indices suggestive of iron deficiency to be present and to contribute to anemia in many subjects. Typical markers of iron deficiency used in CKD are serum ferritin < 100 ng/ml and TSAT < 20%. Clinicians often use these thresholds to base iron treatment decisions, and NKF-K/DOQI guidelines recommend these levels in nondialysis CKD [3]. Specifically, the NKF-K/DOQI guidelines indicate that if either of the above value is low then iron treatment is recommended.

Steven fishbane et al[31] primary finding was that between 57.8 and 72.8% of subjects with CKD have either serum ferritin <100 ng/ml or TSAT <20%. In contrast to these relatively high values of serum ferritin (100 ng/ml) and TSAT (20%) that indicate insufficient iron in CKD, in the

general population lower thresholds of serum ferritin (15 to 30 ng/ml) and TSAT (15%) are often used.

In our study TSAT <20% in 24 cases (44.4%) and serum ferritin <100 ng/ml in 21 cases (38.9%) was present. According to NKF-K/DOQI Guidelines[8] up to 44.4% of patients in our study are in iron deficient state.

In hemodialysis, repeated blood loss makes iron deficiency an almost universal problem (Van wyck et al) [42]. In contrast, in nondialysis CKD, dialysis-related blood loss does not occur, so iron deficiency could occur less frequently. The great prevalence of low iron indices found may not indicate iron deficiency per se, but rather impaired iron delivery concurrent with inflammation, a complex syndrome that occurs with progressive CKD. However, it is plausible that iron deficiency might be more common than expected because in CKD, the prevalence of gastrointestinal pathology with blood loss is probably increased[19], sampling of blood for laboratory testing is common, and hospitalizations and surgery for other intercurrent illness could contribute to blood loss. In addition, many patients are treated with Erythropoeisis Stimulating Agents, further depleting iron stores.

Evidence in support of the high prevalence of iron deficiency that found in CKD is provided by a publication by Gotloib *et al*[32]. These investigators performed sternal bone marrow biopsies on 47 patients with

CKD and Hb < 12 g/dl. Remarkably, severe iron deficiency was found in 46 of 47 subjects, and these patients were subsequently treated with intravenous iron, with most responding with improved Hb concentration[32].

In our study mean serum ferritin was 278.3 ng/ml and 9cases (16.67%) had serum ferritin level >500ng/ml .

Hypothetically, higher amounts of ferritin may trap more body iron and protect the individual against worsening infection, the start of which invariably is associated with inflammation. Hence, inflammation-induced hyperferritinemia may result in a so-called "functional iron deficiency," which may be useful in "acute" inflammation by iron containment in the RES sites but harmful under "chronic" inflammation by leading to refractory anemia such as in CKD or other chronic disease states[27].

Chronic inflammation is common in patients with CKD, and up to 40 to 70% of patients with CKD may have increased C-reactive protein (CRP) levels on a chronic basis. Hence inflammation probably is the most common confounder in CKD-associated hyperferritinemia and may contribute to it more strongly than Iron [44] (Jairam et al)

Hence, a low ferritin level (*e.g.*, 200 ng/ml in hemodialysis patients or 100ng/ml in nondialyzed patients with CKD) is a reliable indicator of iron deficiency, whereas a normal to moderately high serum ferritin does

not rule out iron deficiency or indicate adequate or too much Fe on board[27].

It is well known that occult inflammation is commonly present in CKD and may increase in prevalence with progressive disease. Inflammation has a profound effect on iron indices. Previously, in hemodialysis, CRP, an indicator of inflammation, was found to be highly correlated with serum ferritin values. Anemia in chronic kidney disease is a complex process that reflects an interaction of the erythropoietic processes of bone marrow with iron availability and inflammation.[45, 46] (Landray MJ et al)

In our study we have not done variables measuring the inflammatory state to allow for more effect of inflammation on serum ferritin.

Serum ferritin value was measured in our patients and healthy controls and found to exist a significant difference between them. (P value 0.047). There existed no relationship between Glomerular filtration rate and serum ferritin. As expected those patients with microcytic hypochromic anemia had lower serum ferritin and low transferrin saturation (TSAT) when compared to the normocytic normochromic anemia

This clearly indicates that iron deficiency anemia is a one of the major component in the anemia of chronic kidney disease due to various reasons discussed earlier.

So every effort should be done to identify the cause of anemia in chronic kidney disease patients and treat the coexistent iron deficiency anemia in chronic kidney disease patients and other haematological parameters should be monitored to find out coexisting abnormality.

LIMITATIONS OF THE STUDY

As the study population was small, larger studies are required to validate the results of this study. Iron deficiency in CKD should be assessed by using other newer methods which include soluble transferrin receptors, zinc protoporphyrin, percentage of hypochromic cells, reticulocyte hemoglobin concentration and the gold standard method, the bone marrow examination for stainable iron. Hepcidin level was not done. Also in our patients the probable causes of iron deficiency like occult Gastro intestinal blood loss were not excluded by upper gastrointestinal endoscopy. Folic acid and vitamin B12 assays are not done.

As this was a cross-sectional study, we could not document if the findings were persistent. Finally, this study only shows an association, and cannot prove the causality. Interventional studies will be needed to finally nail down a cause-and-effect relationship.

CONCLUSION

1. In our study, we have found that chronic kidney disease affects predominantly middle aged population.
2. All the patients in this study were in the stage 3, 4 or 5 of Chronic Kidney Disease (CKD).
3. Profound anemia was universal in our patients, which is an important contributor to the high mortality and morbidity in patients with End Stage Renal Disease (ESRD).
4. There was a significant reduction in Hemoglobin, and Packed Cell Volume with progressive increase in severity of CKD stage.
5. Bleeding time increased in 5.6% patients and elevated Erythrocyte Sedimentation Rate was present in more than half of patients.
6. Normocytic normochromic anemia was found in 70.4 % of the patients and microcytic hypochromic anemia in another 20.4%, and 9.2% had both type of morphology in the peripheral smear picture.
7. Patients with microcytic hypochromic anemia had relatively low serum ferritin and low transferrin saturation (TSAT) when compared to the normocytic normochromic anemia patients.
8. Applying the NKF-K/DOQI guidelines for nondialysis chronic kidney disease to our population it was found that nearly 38.9% of

the study population did not have target serum ferritin of 100 ng/ml and 44.4% of study population did not have target TSAT of >20%. So it is vital to address this issue of iron deficiency in patients with chronic kidney disease so that necessary investigations can be undertaken to find the cause of iron deficiency if any.

9. Adequate supplementation of iron should be given either as oral or parenteral route before initiation of dialysis or erythropoietin therapy to attain the goals according NKF guidelines.
10. So every effort should be done to identify the cause of anemia in CKD patients and treat the coexistent iron deficiency anemia in chronic kidney disease patients and other haematological parameters should be monitored to find out coexisting abnormality.
11. Even though treating the complication of CKD like anemia, will reduce the mortality and improve the survival, our ultimate aim should be focused on the preventive strategies for CKD. This include screening high risk population , control of hypertension , DM, limiting the use of nephrotoxic drugs like NSAID'S so that a large section of our population escape the burden of this killer disease.

SUMMARY

This study was undertaken with the aim to assess the Haematological profile and Serum Iron indices in nondialysis chronic kidney disease patients. It is a cross-sectional study conducted in Department of Medicine and Nephrology, Govt Rajaji Hospital.

A total of 54 patients were included in our study who satisfied the diagnostic criteria of CKD and patients underwent clinical and renal parameters, haematological profile and iron status. For comparison of the results with the general population adequate number of controls were also taken.

Haemoglobin, packed cell volume was low with increasing severity of chronic kidney disease. Bleeding time increased in 5.6% patients and elevated ESR was present in more than half of patients. Anemia was universal in our population. Normocytic normochromic anemia was found in 70.4 % of the patients and microcytic hypochromic anemia in another 20.4%, and 9.2% had both type of peripheral smear picture. Applying the NKF-K/DOQI guidelines for nondialysis chronic kidney disease to our population it was found that nearly 38.9% of the study population did not have target serum ferritin of 100 ng/ml and 44.4% of study population did not have target TSAT of >20%. So it is vital to address this issue of iron

deficiency in patients with chronic kidney disease so that necessary investigations can be undertaken to find the cause of iron deficiency if any.

So every effort should be done to identify the cause of anemia in CKD patients and treat the coexistent iron deficiency anemia in chronic kidney disease patients. And other haematological parameters should be monitored to find out coexisting abnormality.

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PROFORMA

S. NO : O.P/I.P. NO

NAME : AGE/SEX:

OCCUPATION :

ADDRESS :

CHIEF COMPLAINTS:

DURATION OF ILLNESS:

H/O Bleeding manifestation

PAST HISTORY:

DM / HT

PERSONAL HISTORY:

Smoking Alcoholism Veg/ Nonveg:

FAMILY HISTORY:

GENERAL EXAMINATION:

VITALS:

PULSE: BP: HEIGHT:

WEIGHT:

SYSTEMIC EXAMINATION:

CVS :

RS :

ABDOMEN:

CNS :

INVESTIGATIONS:

Blood sugar : Urea :

Serum Creatinine: Na+ : K+:

GFR :

COMPLETE HAEMOGRAM:

Hb : TC: DC: P- L- E- M-

RBC : PLATELET: PCV:

MCV : MCHC: MCH:

RDW ESR: BT: CT:

PERIPHERAL SMEAR :

SERUM IRON INDICES:

SERUM IRON :

FERRITIN :

SERUM IRON BINDING CAPACITY:

TRANSFERRIN SATURATION % :

ECG

USG Abdomen:

Others:

Motion occult blood Motion ova cyst

MASTER CHART

S.NO	NAME	AGE	SEX	ILL.DURA	DM	HT	H/O BL	M.O&C	M.OC. BL	ANE	WT	UREA	CREAT	GFR	CKD STAGE	HB	RBC COUNT	PCV	PLT	MCV	MCH	MCHC
1	Amaldoss	50	M	5months	N	N	N	N	N	P	62	68	2.5	31	3	9.8	3.3	28.8	4.38	87.3	29.7	34
2	Immanuvel	59	M	9months	P	P	N	N	N	P	66	92	3	24.75	4	9.1	3.05	29.3	4.09	84.2	31.2	35.4
3	Kandasamy	50	M	6months	N	P	N	N	N	P	62	54	3	25.833	4	9.4	3.66	31.2	3.84	85.2	26.6	32.1
4	Arumugam	28	M	14months	N	P	N	N	N	P	55	179	16.6	5.1539	5	5.6	1.75	16.5	4.66	93.1	32	33.9
5	Suseela	40	F	12months	P	P	N	N	N	P	60	78	3.7	19.14	4	8.8	3.29	28.2	3.89	85.7	26.7	31.2
6	Palanisamy	42	M	10months	N	N	P	N	N	P	58	352	22.9	3.4474	5	6.4	2.28	21.1	4.14	92.5	28.1	30.3
7	Nagarajan	27	M	9months	N	P	N	N	N	P	45	210	8.8	8.0256	5	7.3	3.03	25.6	3.16	84.5	24.1	28.5
8	Chikkaiyappan	43	M	18months	P	P	N	N	N	P	65	149	5.6	15.637	4	5.4	2	18	3.97	74.9	14.4	19.3
9	Mookaiyan	31	M	4months	N	P	N	N	N	P	42	392	12.7	5.0066	5	5.9	4.15	26.8	3.09	82.9	25.2	28.1
10	Palaniyammal	37	F	6months	N	N	N	N	N	P	45	58	2.9	19.12	4	8.2	3.12	27.2	2.75	82.9	26.2	30
11	Vayakattusamy	25	M	11months	N	P	P	N	N	P	48	183	19.6	3.9116	5	3.5	1.89	15.4	2.63	0.43	18.5	22.7
12	Bakiyaraj	29	M	8months	N	P	N	N	N	P	58	111	4.7	19.025	4	8.1	2.85	23.7	3.3	83.2	28.4	34.2
13	Tamilarasan	42	M	7months	N	N	N	N	N	P	66	53	3.2	28.073	4	9.4	3.43	29.2	2.6	85.1	27.4	32.2
14	Samidurai	60	M	9months	N	P	N	N	N	P	57	94	5.6	11.31	5	8.1	3.05	24	2.2	83	30.6	35.8
15	Balasubramaniyan	37	M	5months	N	N	N	N	N	P	65	76	2.8	33.209	3	10	4.15	36.6	3.78	88.2	25.1	28.1
16	Velayutham	53	M	10months	P	P	N	N	N	P	67	104	4	20.24	4	8.6	2.58	23.5	2.7	91.1	33.3	36.6
17	Periyasamy	60	M	8months	N	N	N	N	N	P	65	94	5.6	12.897	5	7.7	4.74	30.4	3.5	64.1	16.2	25.3
18	Mahalingam	50	M	6months	P	P	N	N	N	P	69	102	5.3	16.274	4	8.3	3.37	28.5	2.5	84.6	24.6	29.1
19	Veeranan	55	M	5months	P	P	N	N	N	P	63	58	3.5	21.25	4	11	3.41	29.2	2.2	85.6	28.7	33.6
20	Karuppaiya	59	M	12months	N	P	N	N	N	P	54	140	11.1	5.473	5	4.8	4.65	26.6	2.41	83	29.4	32.8
21	Murugesan	45	M	15months	P	N	N	N	N	P	46	109	7.9	7.6828	5	7.2	2.52	21.1	3.18	83.7	28.6	34.1
22	Paramasivam	57	M	6months	N	N	N	N	N	P	70	168	11.6	6.9564	5	7.3	3.42	23.7	2.5	86.3	27.1	30.7
23	Ramayee	20	F	3months	N	P	N	N	N	P	43	78	3.3	18.45	5	9.8	3.04	28.9	3.72	95	32.2	33.9
24	Karuppusamy	40	M	9months	N	P	N	P	N	P	60	99	14.4	5.787	5	6	3	19.4	2.98	65	20	30.9

25	Marimuthu	58	M	12months	P	P	N	N	N	P	70	227	11.3	7.0551	5	7.7	2.83	23.6	2.62	83.4	27.2	32.6
26	Lakshmanan	21	M	8months	N	N	N	N	N	P	36	127	8	7.4375	5	7.5	2.89	25	2.4	86.5	26	30
27	Meenakshi	28	F	15months	N	P	N	N	N	P	48	112	6.4	9.92	5	8.2	3.19	26.9	4.33	84.3	25.7	30.5
28	Alagarsamy	49	M	6months	N	N	N	N	N	P	69	78	2.1	41.528	3	7	4.31	26.8	3.34	62.3	16.2	26
29	Valarmathi	53	F	5months	N	N	N	N	N	P	54	206	7.4	7.49	5	5.7	2.61	21.4	3.22	82	21.8	26.6
30	Abdul jaffer	44	M	10months	N	P	N	N	P	P	62	140	4.2	19.683	4	8.8	4.22	28.2	3.7	66.8	20.9	31.2
31	Kowsalyastella	58	F	12months	N	N	N	N	N	P	69	236	8	8.35	5	9.8	3.3	28.8	3.26	87.3	29.7	34
32	Ochammal	53	F	6months	P	P	N	N	N	P	52	178	5.2	10.27	5	8.7	3.02	25.3	3.7	83.8	28.8	34.4
33	Janarthanan	49	M	8months	N	N	N	N	N	P	65	192	6.9	11.906	5	9.4	2.94	26	3.67	88.4	33	37.3
34	Thevendran	38	M	6months	N	N	N	N	N	P	49	216	8	8.6771	5	7.5	3.22	28.3	2.79	87.9	23.3	26.5
35	Kathamuthu	51	M	12months	N	P	N	N	N	P	57	82	2.5	28.183	4	8.9	2.85	26.3	3.3	92.3	31.2	33.8
36	Raajeswari	42	F	15months	N	P	N	N	N	P	55	96	3	21.21	4	8.3	2.34	21.9	4.2	93.6	33.5	36
37	Chinnaiyan	57	M	24months	N	N	N	N	N	P	50	142	3.9	14.779	5	8.2	2.6	22.9	2.97	88.1	31.5	35.8
38	Janaki	40	F	6months	N	N	N	N	N	P	45	124	3.5	15.18	4	9.3	3.34	30.1	3.2	90.1	27.8	30.9
39	Subbaiya	55	M	4months	N	N	N	N	N	P	49	76	1.9	30.446	3	9.4	2.69	23.4	2.79	87	31.8	35.7
40	Patchamuthu	47	M	3months	N	N	N	N	N	P	60	82	2	38.75	3	9.9	3.24	28.7	2.65	88.6	30.6	34.5
41	Pandiyammal	39	F	6months	N	P	N	N	N	P	51	104	3.5	17.37	4	3.5	1.89	15.4	4.12	81.5	18.5	22.5
42	Duraisamy	50	M	3months	N	N	N	N	N	P	57	68	2.1	33.929	3	9.6	3.35	28.1	3.47	83.9	28.7	34.2
43	Abdulla	19	M	6months	N	N	N	N	N	P	30	127	8	6.3021	5	7.7	2.83	23.6	2.75	83.4	27.2	32.6
44	Muthupandi	57	M	12months	N	N	N	P	N	P	38	156	20.9	2.096	5	4.9	2.96	18	3.11	60	16	27.2
45	Pandiyan	38	M	8months	N	P	N	N	N	P	49	96	2.1	33.056	3	9	2.8	23.3	2.47	83.2	32.1	38.6
46	Kumaravelan	23	M	6months	N	N	N	N	N	P	43	189	3.8	18.388	4	8.1	4.08	29.9	3.97	73.3	19.9	27.1
47	Alagarsamy	44	M	5months	P	N	N	N	N	P	54	67	1.6	45	3	11	3.66	32	2.87	87	30.3	34.7
48	Ramasamy	52	M	12months	P	P	N	N	N	P	59	98	2.2	32.778	3	8.8	2.92	24.3	3.2	83.2	30.1	36.2
49	Perikaruppan	57	M	12months	N	N	N	N	N	P	65	107	3	24.977	4	9.4	4.41	30.1	4.1	68.2	21.4	31.4
50	Kalpana	26	F	4months	N	P	N	N	N	P	42	165	4.2	15.833	4	6	4.31	26.8	4.45	62.3	16.2	26
51	Ganesan	40	M	6months	N	N	N	N	N	P	60	202	6.7	12.438	5	5.8	2.35	20.6	4.56	87.7	24.7	28.2
52	Selvaraj	35	M	9months	N	N	N	N	N	P	59	64	1.8	47.801	3	9.8	3.3	28.8	2.18	87.3	29.7	34
53	Subramani	43	M	12months	N	P	N	N	N	P	62	88	1.9	43.962	3	9.7	4.12	28.9	4.48	70.1	23.5	33.6
54	Selvi	31	F	3months	N	N	N	N	N	P	49	148	2.4	30.909	3	6.3	3.54	24.9	3.79	69.7	20.7	29.7

S,No.	RDW	ESR	BT	CT	WBC	N	L	E	M	P.S	LVH	IRON	TIBC	TSAT	FERRITIN
1	14.6	14	nl	nl	9600	66	30	3	1	nn	N	79	286	27.62	148
2	14	30	nl	nl	7100	64	23	6	7	nn	N	72	356	20.22	96.2
3	13.6	12	nl	nl	8100	55	39	4	2	nn	N	44	356	12.35	59.9
4	13.5	70	nl	nl	8200	58	39	2	1	nn	P	12	219	5.48	1245.2
5	12	18	nl	nl	5800	52	45	3		nn	N	39.7	100	39.7	262
6	14.2	38	INCR	nl	7000	58	39	3		nn	P	119.7	188	63.67	586.4
7	13.8	40	nl	nl	6900	60	38	2		nn	N	197.4	249	79.25	424.9
8	31.9	44	nl	nl	6100	58	36	2	2	mh	P	43	372	11.56	27
9	13.8	64	nl	nl	5900	62	37	1		nn	P	133.8	160	83.63	1880
10	13.7	28	nl	nl	6500	65	30	4	1	nn	N	56	280	20	890
11	16.3	36	INCR	nl	9700	51	43	3	3	both	P	64	380	16.84	63.8
12	13	60	nl	nl	8100	59	34	4	3	nn	N	102	402	25.37	298
13	12.9	30	nl	nl	7300	60	32	4	4	nn	N	49	360	13.61	32.1
14	14.2	36	nl	nl	8300	63	32	3	2	nn	N	126	367	34.33	532.4
15	14.9	14	nl	nl	9300	54	41	3	2	nn	N	82	322	25.47	435
16	14.2	34	nl	nl	7300	60	31	8	1	nn	N	96.2	290	33.1	146
17	24.5	20	nl	nl	5800	65	32	3	2	mh	P	38	381	9.97	24.3
18	14.6	70	nl	nl	6300	57	40	2		nn	N	134	288	46.53	836.1
19	14.5	15	nl	nl	7000	42	49	4	5	nn	N	51.3	270	18.89	146.3
20	13.8	45	nl	nl	7000	54	40	3	3	nn	P	58	336	17.26	38.5
21	14.2	20	nl	nl	7400	67	30	3		nn	P	47	361	13.1	279.4
22	14.1	25	nl	nl	6400	64	30	2	4	nn	P	33	135	24.44	307
23	13.2	46	nl	nl	7300	62	32	3	3	nn	N	54	299	18.06	243
24	16.6	70	nl	nl	6000	76	20	3	1	mh	P	43	359	11.97	42.7

25	14.4	30	nl	nl	7100	60	36	4		nn	N	62	192	32.29	187.2
26	12.9	15	nl	nl	7500	67	30	3		nn	P	76.3	234	32.4	204.7
27	13.6	18	nl	nl	8100	62	32	2	2	nn	N	88	302	29.14	193.6
28	22.6	36	nl	nl	8800	40	53	4	3	mh	P	40	392	10.2	27.5
29	19.9	19	nl	nl	6700	65	32	4	1	both	P	49	334	14.67	33.9
30	19.3	15	nl	nl	5800	67	30	3		mh	N	53	390	13.59	19.2
31	14.4	20	nl	nl	6900	62	34	3	1	nn	N	92.7	210	44.14	508.2
32	13.7	18	nl	nl	4800	61	31	8		nn	P	88	218	40.37	322
33	13.5	20	nl	nl	5300	57	40	3		nn	N	101	305	33.11	664.8
34	16.2	14	nl	nl	6400	63	32	2	3	both	P	45	367	12.26	38.2
35	14.2	14	nl	nl	7900	64	29	3	4	nn	N	70.2	206	33.98	232.9
36	13.8	22	nl	nl	7100	55	39	4	2	nn	N	86	189	45.5	164
37	14.2	8	nl	nl	8200	51	44	3	2	nn	N	68	187	36.36	421
38	14.4	20	nl	nl	6900	64	33	3	2	nn	N	89	204	43.62	258.7
39	12.7	10	nl	nl	6100	60	36	3	1	nn	N	92	221	41.62	453.6
40	13	14	nl	nl	5800	52	38	6	4	nn	N	67	176	38.07	397.2
41	18.7	18	nl	nl	7800	59	35	5	1	both	P	43.1	348	12.35	18.5
42	12.5	12	nl	nl	6400	72	26	2		nn	N	65	168	38.69	327.5
43	13.4	36	nl	nl	5000	58	35	5	2	nn	P	46	297	15.49	658.9
44	14.1	40	INCR	nl	7200	56	39	4	1	mh	N	39	339	11.5	24.2
45	13.9	12	nl	nl	7100	58	40	1	1	nn	N	61.2	210	29.08	244
46	26.9	34	nl	nl	9600	59	35	5	1	mh	N	40	335	11.94	9.7
47	16.5	10	nl	nl	8000	66	29	1	4	both	N	49	322	15.21	64.7
48	14.3	14	nl	nl	5700	57	35	3	5	nn	P	72.7	309	23.03	128.3
49	18.8	12	nl	nl	7100	71	18	8	3	mh	N	53	375	14.13	19.4
50	22.6	20	nl	nl	5200	64	32	1	3	mh	P	39	289	13.49	57.8
51	13	26	nl	nl	5900	75	17	5	3	nn	P	84	269	31.23	164.1
52	13.6	10	nl	nl	6700	67	26	5	2	nn	P	66	280	23.57	98.4
53	25.8	14	nl	nl	9300	63	33	1	3	mh	N	59	349	16.9	27.8
54	26.7	18	nl	nl	4700	62	33	2	3	mh	P	52	388	13.4	12.8

KEY TO MASTER CHART

Sex	-	M - Male F - Female
ILL.DURATION	-	Illness duration
DM	-	Diabetes mellitus
HT	-	Hypertension
H/O BL	-	History of bleeding
M.OC.BL	-	Motion for occult blood test
ANE	-	Anemia WT - Weight
CREAT	-	Creatinine
GFR	-	Glomerular Filtration Rate
HB	-	Hemoglobin
PCV	-	Packed cell volume
PLT	-	Platelet
MCV	-	Mean corpuscular volume;
MCH	-	Mean corpuscular hemoglobin
MCHC	-	Mean corpuscular haemoglobin concentration
RDW	-	Red cell distribution width
ESR	-	Erythrocyte sedimentation rate
BT	-	Bleeding time
CT	-	Clotting time
WBC	-	White cell count
N	-	Neutrophil;
L	-	Lymphocyte
E	-	Eosinophil
M	-	Monocyte
P.S	-	Peripheral smear
LVH	-	Left ventricular hypertrophy
%TSAT	-	% Transferrin saturation